Sperm, a Source of Estrogen

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This review article discusses a novel nontraditional site of estrogen synthesis and the potential targets of estrogen action within the male reproductive system. Our laboratories have recently demonstrated that developing spermatids in several species contain aromatase, the cytochrome P450 enzyme responsible for converting androgens into estrogens. The enzyme was localized by immunocytochemistry and the protein's presence was confirmed by Western blot analysis. Northern blot analysis and *in situ* hybridization were used to corroborate the presence of mRNA for aromatase. It appears that the aromatase message precedes the synthesis of the protein, and the protein remains in the spermatids several days after the message disappears. The enzyme is located along the tail of newly released sperm and is active in the epididymal sperm as well as in the developing germ cells of the testis. This unique discovery is the basis for our overall hypothesis that estrogen, synthesized by sperm, plays a role in the regulation of epididymal function proportional to the number of sperm being transported. The presence of an estrogen source within the ductal lumen is of special importance to the study of epididymal function because the regulatory mechanisms in this region remain unclear, particularly for the efferent ductules and initial segment regions, although estrogen receptors have been identified in the ductal epithelium. An understanding of the role that estrogen plays in the function of the epididymis may provide benefits in several areas including the treatment of abnormalities in epididymal function, the potential development of a male contraceptive, and insight into the causes of adult epididymal lesions induced by neonatal exposure to estrogenic compounds such as diethylstilbestrol. — Environ Health Perspect 103(Suppl 7):59–62 (1995)

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Introduction

Estrogen is typically considered the female hormone. Its synthesis in the male has not been emphasized because of the importance of testosterone, the androgen responsible for male reproductive structure and function. It has been known for several years that the testis synthesizes estrogen as well as testosterone (*1,2*). In fact, rete testis fluid of the rat has an estrogen concentration of nearly 250 pg/ml, which is relatively high compared to blood plasma (*3*). Even though the presence of estrogen is acknowledged in the male reproductive system, its function and targets in the male are not understood. There is little information regarding estrogen-responsive genes or proteins in the testis or male reproductive tract. In this brief paper, we review the data from our laboratories showing that male germ cells are capable of synthesizing estrogen. We also discuss the potential nontraditional sites of estrogen action within the male reproductive system.

The testis is composed of two compartments, the seminiferous tubules and the interstitial space between the tubules. These two compartments include only three major cell types. The germinal cells are surrounded and nurtured by the cytoplasm of the Sertoli cells within the seminiferous tubules. Leydig cells, which synthesize testosterone, are present only in the interstitial space. The primary enzyme involved in testicular steroidogenesis, 3β-HSD (hydroxysteroid dehydrogenase), is a marker for the Leydig cell's functional capacity. However, it has been traditionally accepted that in the adult male the Leydig cell also synthesizes estrogen, whereas in the immature animal it is the Sertoli cell that is responsible for estrogen synthesis (*4–8*).

Much of the work supporting these traditional sites of estrogen synthesis was performed before the establishment of purified cell preparations from the germinal epithelium and before the purification of cytochrome P450 aromatase (P450arom), the enzyme responsible for the conversion of androgens to estrogens. Thus, it is not surprising that there is considerable controversy in the literature over the localization of P450arom in the male (*2,9*). Therefore, our purpose was to examine the localization of P450arom in the whole testis as well as in isolated testicular cells and epididymal sperm.

Aromatase in the Testis

To localize aromatase in the male reproductive system, we have taken a multifaceted approach, beginning first with immunocytochemistry (ICC), which was confirmed by Western blot analysis. The ICC was followed by Northern blot analysis to determine that the mRNA was indeed present in specific cell types of the testis. Finally, the activity of aromatase in isolated germ cells was determined using the ^3H_2O assay.

ICC and Western analysis were performed using an affinity-purified antibody to human placental P450arom developed by Osawa (*10*). In our first study, aromatase was localized in the adult mouse testis (*11*). It was not surprising to find that the Leydig cells stained positive for aromatase. However, to our surprise, the seminiferous tubules were also positive, with a prominent staining near the luminal surface. At higher magnification the Leydig cells were clearly stained, but spermatids within the seminiferous epithelium were stained even more intensely. Thus, using ICC we presented the first evidence that male germinal cells contain aromatase. Because spermatogenesis is divided into stages of development based upon specific cellular associations (*12*), we were then able to follow clearly the appearance of aromatase within the germ cells. The data

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Abbreviations used: 3β-HSD, hydroxysteroid dehydrogenase; P450arom, P450 aromatase; ICC, immunocytochemistry; kb, kilobases; DES, diethylstilbestrol.

showed that aromatase was present first within the Golgi system of the early round spermatids at approximately stage II. As the Golgi apparatus migrated away from the nucleus toward the forming tail of the spermatid, aromatase became more dispersed within the cytoplasm. Finally, as the sperm tail formed, the Golgi disappeared but aromatase remained within the cytoplasm and intensified along the tail of the developing spermatids.

The ICC results in the mouse have now been extended to several other species including the rat (*13*), the bear (*14*), and the chicken (*15*). Although ICC localization indicated that aromatase is present in developing spermatids, one must be cautious until the results are confirmed by Western and Northern blot analyses. Therefore, cells were isolated from the testis of the mouse and the rat using the Sta-Put method, and total cellular protein or total RNA was obtained for analysis. By Western analysis a prominent 55-kDa protein in pachytene spermatocytes and round spermatids co-migrated with the purified human placental P450arom (*11,16*). Aromatase mRNA has been confirmed in mouse testis, pachytene spermatocytes, and round spermatids using Northern blot analysis (*11*). Three transcripts were identified at 3.5, 3.1, 2.5, and 1.8 kb, two of which are similar in size to the mouse ovary aromatase message (*17*). Preliminary *in situ* hybridization data also indicated that the aromatase message is present beginning in the late spermatocytes and continuing in the round spermatids. Using the STAGES program (*18*), it was calculated from these data that the message precedes the protein appearance in the Golgi region of the round spermatids by approximately 6 days and that the protein continues in the absence of the message for approximately 12 days.

Although we had clearly shown that spermatocytes and spermatids produce the aromatase mRNA and its protein, there remained an important question: Do germ cells of the testis actually exhibit aromatase activity? To answer this question, we determined the activity in microsomal preparations of isolated testicular cells from the mouse (*11*) and the rat (*16*) using the ^3H_2O assay (*19*). In this assay, 1 mol of ^3H_2O is released by the aromatization of 1 mol of labeled androstenedione. It is noteworthy that the germ cell preparation showed nearly 35% greater activity than the interstitial cells. Thus, these data are consistent with that of the ICC in which the mouse spermatids stained more intensely than the Leydig cells. Moreover, Northern blots showed a stronger signal in the isolated cell types than in the mixed germ cell preps.

Aromatase in Epididymal Sperm

The data collected from studies of testicular aromatase demonstrated that the developing male germ cells, like the Leydig cells, have the capacity to synthesize estrogen. However, it remained to be determined whether mature sperm contained aromatase as they left the testis. Therefore, sperm were collected from two regions of the epididymis: efferent ductules and caput, corpus, and cauda epididymides. Aromatase activity was determined in microsomal preparations of the isolated sperm. It was found that in both mouse and rat, epididymal sperm aromatase activity was present although less active than that seen in testicular germ cells. Interestingly, the activity was differentially present with greater activity in sperm taken from the caput region, and activity declined as sperm traversed the male reproductive tract from caput to cauda epididymis (*16,20*). ICC was also used to localize aromatase in the epididymal sperm. Data showed that sperm tails in the efferent ductules and caput epididymis were more positively stained than were sperm in the more distal regions of the tract. Thus, these data indicate that epididymal sperm may be one of the sources of estrogen that is present in substantial amounts within the rete testis fluid.

Potential Estrogen Target Sites in the Male Reproductive System

The preceding data have led to the development of the following overall hypothesis:

> Estrogen, synthesized by sperm-associated P450 aromatase, plays a role in the regulation of epididymal function proportional to the number of sperm being transported.

Use of the general term epididymis here includes the efferent ductules (ductuli efferentes) that connect rete testis and initial segment epididymidis.

This hypothesis is supported by our data showing that developing spermatids in the mouse and rat testis express aromatase and that this enzyme is active both in the germ cells of the testis and the sperm of the epididymis (*11,16,20*). In addition to these data, this hypothesis is further supported by several lines of reasoning:

- There is evidence that estrogen receptors are present in the head of the epididymis (Table 1).
- The head of the epididymis is not maintained by circulating androgens (*21*) but may be dependent upon sperm-associated factor(s) present in the rete testis fluid (*22*).
- The reabsorption of luminal fluids should be proportional to the number of spermatozoa being transported; thus, if fluid reabsorption is inhibited, intraluminal sperm become diluted (*23*); conversely, if reabsorption is stimulated, sperm can become compacted and form ductal occlusions (*24–26*).
- It is unlikely that androgens alone can provide the precise control needed for ductal function due to the high concentration of circulating androgens that is maintained in the male, especially in the efferent ductules, which lack 5α-reductase.
- It is anticipated that only minuscule amounts of estrogen within the ductal lumen are required to alter epithelial function because, in general, estrogen is highly potent at very low concentrations.
- Tamoxifen, an antiestrogen used in the treatment of men exhibiting oligospermia, increases sperm concentration (*27*), which suggests an effect on fluid production or reabsorption.
- It should also be noted that the putative cell types that have been implicated in the reabsorption of ions, water, or proteins (principal cells and apical cells) and cytoplasmic droplet material (clear cells) are the cells that also appear to contain estrogen receptors.

Based upon these arguments, it appears that the function of estrogen in the male will be found primarily in the epididymis. However, this does not preclude the possibility for an estrogen function directly in the testis. There are little data to support

Table 1. Estrogen receptors in the male reproductive system.

		Fetal	Perinatal	Adult
Testis	Sertoli cell	+	–	–/+
	Leydig cell	+	+	+
Ductuli efferentes	Epithelium	+	+	+
	Connective tissue	+	–	–
Epididymis	Epithelium	+	+	+
	Connective tissue	+	+	–/+
Prostate	Epithelium	–	–	+/–
	Connective tissue	+	+	–/+

From Stumpt et al. (*34*), Schleicher et al. (*35*), West et al. (*36*), West and Brenner (*37*), Iquchi et al. (*38*), Greco et al. (*39*), Cooke (*40*), and Sato et al. (*41*).

such a hypothesis, but a recent paper has shown that estrogen does modulate the expression of cadherin in the developing testis (28). It is intriguing to postulate that estrogen may play a role in the regulation of the stage-specific expression proteins during spermatogenesis; however, there are no data to support this idea at this time.

Future studies of estrogen synthesis and function in the epididymis are significant because the head of the epididymis is frequently affected in cases of male infertility (*29*). However, there have been few studies designed to identify the role of estrogen in male reproductive tissues, and its specific action "is still poorly understood..." (*30*). In a recent paper, Lubahn et al. (*31*) utilized gene-knockout technology to demonstrate the importance of estrogen receptor in male and female mice. Their study showed that, in males lacking normal estrogen receptors, the male reproductive system developed but the testis was small in size, and the males were basically infertile.

An understanding of the role of estrogen in the function of the epididymis may provide benefits in several areas, including the treatment of abnormalities in epididymal function, the potential development of a male contraceptive, and insight into the causes of adult epididymal lesions induced by neonatal exposure to estrogenic compounds such as DES (*32,33*).

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